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Effect of water stress on proline accumulation and stomatal resistance in durum wheat (*Triticum durum* Desf.)

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Abstract

In order to understand the effect of water stress and try to select resistant durum wheat genotypes, the present study was conducted on four leaf stage seedlings (parents and F1 hybrids) which were submitted, under semi-controlled conditions, at different stress levels 40, 30, 20, 10 and 6.5% of field capacity (F.C.). The parameters studied are the proline dosage, the stomatal resistance measurement and the heterosis calculation. The results show positive correlations between stomatal resistance and proline accumulation, on the one hand, and the different levels of water stress applied, on the other hand. Under severe water stress, 6.5% F.C., the proline content is in the majority of genotypes, 96 to 246 times the value recorded under basic stress, 40% F.C. With the exception of the hybrid Dek x Hau, it is in the order of 723. The most significant concentrations are recorded in F1 hybrids while parents take intermediate levels. Stomatal resistance increases with the degree of stress and stress is no different really than stress 10% F.C. Hybrid vigor is manifested clearly in the F1. The behavior of genotypes is not associated with an isolated physiological behavior of the variety, but a strategy that includes one or more mechanisms of tolerance and (or) avoidance of water stress.

Keywords: Durum wheat, water stress, proline, stomatal resistance, tolerance.

Introduction

Grains play a undeniable nutritional and economic role. In Algeria, cereal crops occupy areas located in arid and semi arid areas. Algeria's climate is characterized by episodes of water deficit and high temperatures that can occur, a sudden or gradually at the beginning, middle or end of the season. These droughts are sometimes intense, always unpredictable and variable from year to year. This irregularity made that the climate of a region is highly variable, therefore the production of rainfed crops, especially cereals will vary over the years. To improve the production and make it more stable, more routes have been followed including research and creation of new varieties more adapted and more resistant to such conditions. However, this research requires analysis and understanding of different modes of resistance developed by plants to identify selection criteria that can be used in breeding programs.

The drought resistance has been associated with several characteristics of phenological, morphological, physiological and biochemical order reflecting different types of adaptation: Evasion, avoidance or tolerance (Turner, 1979). As part of an integrated pest management, the benefits of genetic improvement (Advances in Physiology) in understanding the adaptive mechanisms (large untapped genetic variability and the use of sound methods of selection) should enable it to play an

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important role, creating new varieties better adapted to drought conditions.

The aim of this work is to create new durum varieties resistant to water stress, or a genetic study was conducted on parents and their F1 descendants, whose ideotype is based on accumulation and proline and measure of stomatal resistance.

Materials and methods

Plant material

The study involved nine durum wheat genotypes: five parents and four hybrids (F1 progeny). Parents are varieties from local and exotic (Table 1). The hybrid genotypes from the cross of her parents who are: (Hed x Vit), (Hau x Hed), (Hed x INRAT), and (Dek x Hau).

Performing the test

The grains were sown in pots filled with 2 kg of clay loam agricultural soil from the nursery of Beekeeping Centre Chaabet El shaved, Constantine with an average of 6 seeds per pot and three replicates for each genotype. Irrigated by water which is the fourth of the field capacity, the experiment was conducted in a small chamber and under semi-controlled conditions with a long day photoperiod of 16 h (8000 lux brightness) and a temperature between 21 and 45°C.

Stress is applied to the four-leaf stage by stopping irrigation until the stress levels of 40, 30, 20, 10 and 6.5% of field capacity by daily measurement of the pots with a balance of type (Avery Berkel FX 220.Max 30kg. 100g Min. Error=5 g).

Parameters studied

The parameters studied are proline, stomatal resistance and calculation of heterosis. Proline was assayed by the method of Troll and Lindsley (1955) modified by Dreier and Corning (1974). Stomatal resistance (noted RS) is measured by a type Prometra AP4 (Tasca in Rashed, 1995) using the formula: **RS=(Rfs+Rfi)/2** where Rsf corresponds to the resistance of the upper side of the leaves, and **Rfi** corresponds to the resistance of the underside of the leaves.

Heterosis (noted H) was estimated for the F1 hybrids using the equation: H = (h - MP) / MP with MP = (P1 + P2) / 2. h = medium hybrid; MP = Average parents, P1 = mean of female parent, P2 = mean of male parent. The results are the average of three repetitions.

The statistical study is led by analysis of variance with two factors, with the Statitcf software.

Table 1. Range, origin, and characteristics of durum wheat varieties (parents) studied.

Parents	Origin	Characteristics
Hedba3 (Hed)	Algeria (I.T.G.C.)	Average productivity, late tillering medium, medium height, drought tolerant
Djnah el khetaifa (Dek)	Algeria (I.T.G.C.)	susceptible to disease, semi-late tillering medium, few drought tolerant
Vitron (Vit)	Spain	Productive, early tillering medium, short, somewhat drought tolerant
Haurani (Hau)	Syria	Semi-Syria early tillering low, short, drought tolerant
INRAT69 (INRAT)	Tunisia	Productive, semi-early tillering medium to high, low height, drought tolerant

Table 2. Range of durum wheat hybrids obtained.

Hybridation	Parent Females (P1)	Hybrid (H)	Parent male (P2)
Hed x Vit	Hed	Hed x Vit	Vit
Hed x Hau	Hed	Hed x Hau	Hau
Hed x INRAT	Hed	Hed x INRAT	INRAT
Dek x Hau	Dek	Dek x Hau	Hau

Results and Discussion

Proline content

The proline content was estimated from the stress level of 40% field capacity (FC). Given the work Chaib (1998), Malki (2002), Redjamia (2006) and Zarafa (2006), the accumulation begins only at the level of 40% C.C. where the obtained content is less than 2 µmol/mg DM than that found by (Benlaribi and Monneveux, 1988) under non-limiting irrigation conditions.

Proline content in leaves of durum wheat grows proportionally to the reduction of soil water content. This positive correlation between these two parameters was observed in durum wheat (Benlaribi and Monneveux, 1988; Chaib, 1998), wheat (Riazi al. 1985; Monneveux and Nemmar, 1986), and barley (Lwin *et al*., 1978; Stewart *et al.,* 1978). It is also observed in other types of osmotic stress (light and heat) Dörflinger and Askman, 1989; Oben and Sharp, 1994; Kanouni and Alatou, 2006) and under salt stress in leaves Tomato (Zid and Grignon, 1991). In the case of 40% F.C treatment, the proline content is 0.26 µomol / mg DM. This content is very low in he leaves and the productive organs under favorable conditions (Kneu and Chen, 1986).

Hybrids Hed x Hau and Hed x INRAT give higher levels grades to those of their biological parents, with a gain of 18.50% and heterosis of 22.72% in turn. While the hybrid Hed x Vit has an intermediate value between the two biological parents, with a slight depression of 6%. On the other hand, hybrid Dek x Hau has a value of accumulation below their biological parents expressed a strong loss equivalent to 72% (Figure 1).

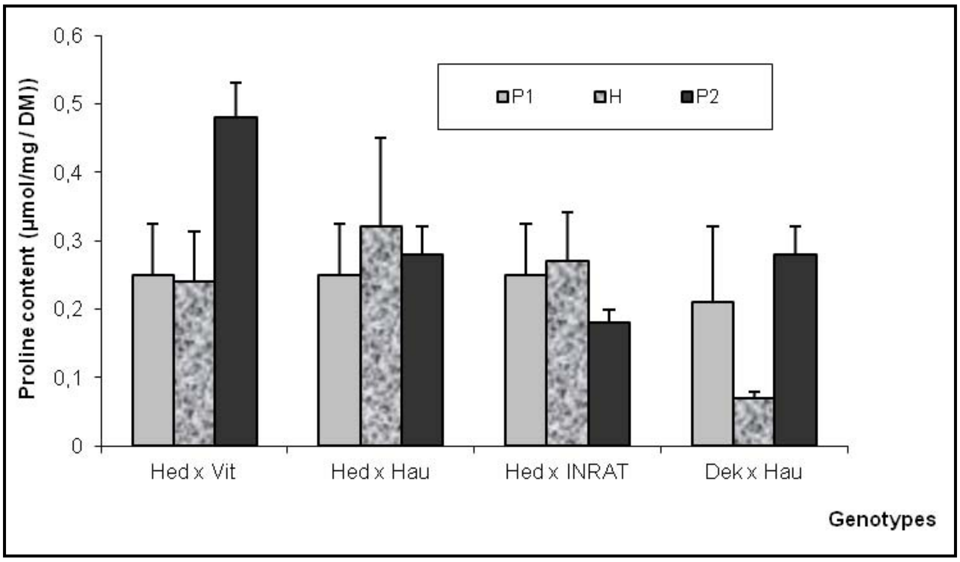

Figure 1. Leaves proline content in nine durum wheat genotypes (parents and F1 hybrids) under treatment of 40% FC.

Under the 30% FC treatment, the proline content is 3 to 6 times higher in the majority of our genotypes compared to that found in the case of 40% FC treatment (Figure 2). It is of the order of 3 times with both parents Vit and Hau, 6 times for parents Dek and 4 to 5 times in all hybrids and the parent INRAT. While the hybrid Dek x Hau reached a maximum of 26 times. All hybrids record levels above those recorded at home parents where hybrid vigor is well illustrated in F1. These gains are respectively 14, 26 , 27, and 59% for the four hybrids Hed x Vit, Hed x INRAT, Hed x Hau and Dek x Hau .

Under moderate water stress, 20% FC, a marked increase is shown in all parental and hybrid genotypes in the range of from 21 to 47 times (Figure 3). It reaches 116 times the initial value at Dek x Hau. Our results are consistent with other researchers in similar conditions. The order of magnitude in durum wheat is 25 times the base value (Benlaribi and Monneuveux, 1988), from 19 to 48 times (Chaib and Benlaribi, 2006a), 30-85 times (Radjamia, 2006). It is the order of 38 times in sunflower (Navari *et al*., 1992), and from 10 to 25-fold in *Vicia faba* L. (Venekamp and Koot, 1988).

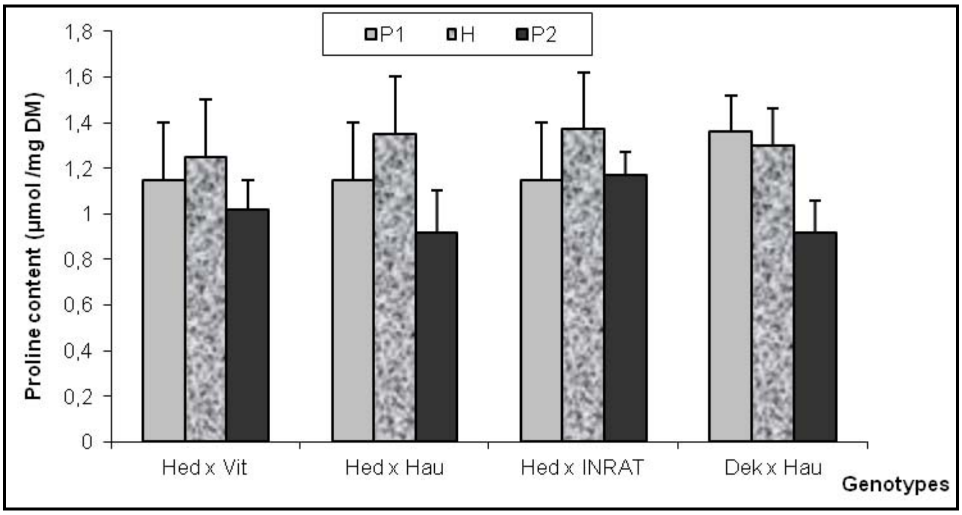

Figure 2. Leaves proline content in nine durum wheat genotypes (parents and F1 hybrids) under treatment of 30% FC.

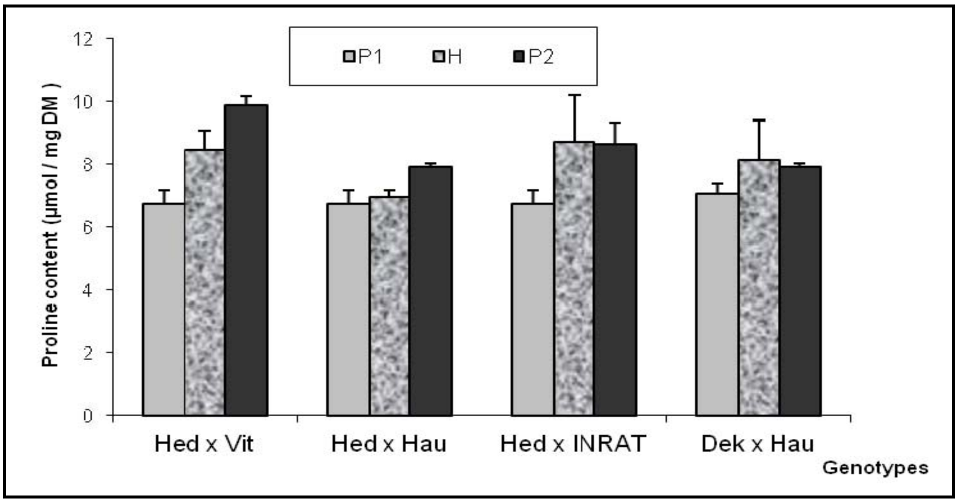

Figure 3. Leaves proline content in nine durum wheat genotypes (parents and F1 hybrid) under treatment of 20% FC.

Monneveux and Nemmar (1986) concluded that this accumulation is closely linked to lack of water and high temperatures. The measurements lead to the conclusion that the genotypes that feel the installation of water deficit react with an increase in proline content in their leaf tissues. At this stage, begins the discriminated of the hybrid of his parents which is manifested by accumulation of higher or lower proline levels compared to those of his parents. The two best hybrids, Hed x INRAT and Dk x Hau, are distinguished by improved content to their parents, with a gain of 24.02 and 8.27% successively. While, the hybrid Hed x Vit is characterized by a slight gain that is negligible of 1.56%. On the other hand, the hybrid Hed x Hau loses 5.04% of its hybrid vigor.

Looking at the evolution of proline accumulation between stress 40 and 20% FC (Figures 1, 2 and 3), we can suggest that this rapid proline accumulation from the beginning of the water deficit, would contribute in maintaining the pressure turgor. According to Riazi *et al.* (1985), increased levels of proline did not occur until after osmotic adjustment.

Under severe water stress, 10% FC, the accentuation of the accumulation of proline is triggered in all genotypes. It is of the order of the order of 52 to 106 times in the majority of genotypes compared to the values obtained with a stress of 40% FC (Figure 4). However the hybrid Dek x Hau reaches an accumulation rate of 597 times. The three hybrids Dek x Hau, Hed x Hau and Hed x INRAT contain high levels compared to their biological parents with a maximum improvement of heterosis equal to 206, 120 and 38% respectively. While the hybrid Hed x Vit h as content between the two biological parents with a loss of 40%.

At the end of the experiment, a very severe stress 6.5% FC, triggers allows to pursue and achieve a maximum of proline content (Figure 5). The accrual rate is, in the majority of genotypes, approximately 96 to 246 times the initial value unregistered in the case of 40% FC. Only the hybrid Dek x Hau from two tolerant parents registered an increase of 723 times the base value. The four levels of F1 hybrids were improved compared to their biological parents with a maximum improvement of hybrid vigor reaching 100% (Gallais, 1989).

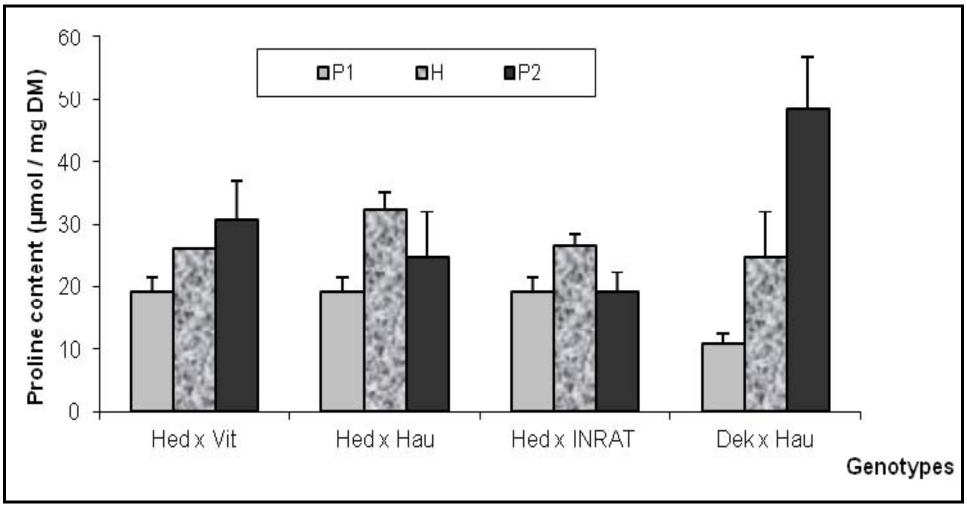

Figure 4. Leaves proline content in nine durum wheat genotypes (parents and F1 hybrid) under treatment of 10% FC.

The gain of heterosis is 54, 59, 63 and 100% in four of the F1 hybrids Hed x Vit, Hed x Hau, Hed x INRAT and Dek x Hau respectively. The content of this treatment really argues the effect stress (low water) on the accumulation of proline to hybrids and their parents.

These results are consistent with those reported by Chaib and Benlaribi (2006b) and Radjamia (2006). This increased flow of proline is a phenomenon of drought adaptation allows the plant to maintain its turgor by decreasing the osmotic potential (Monneveux, 1991). Proline acts as an osmoticum related to the level of stress tolerance (Singh *et al*., 1973; Stewart *et al*., 1974; Kauss, 1977). This tolerance allows the plant to ensure its physiological functions, despite a deterioration of its internal water statue

(De Raissac, 1992). It could also be involved in the regulation of cytoplasmic pH (Pesci and Befagna, 1984) and create a pipeline of nitrogen used by plants in the later periods of stress (Tall and Rosenthal, 1979). At the end of stress (6.5% FC), the four hybrids (Figure 5) provide the best content. They present the maximum positive notion of hybrid vigor.

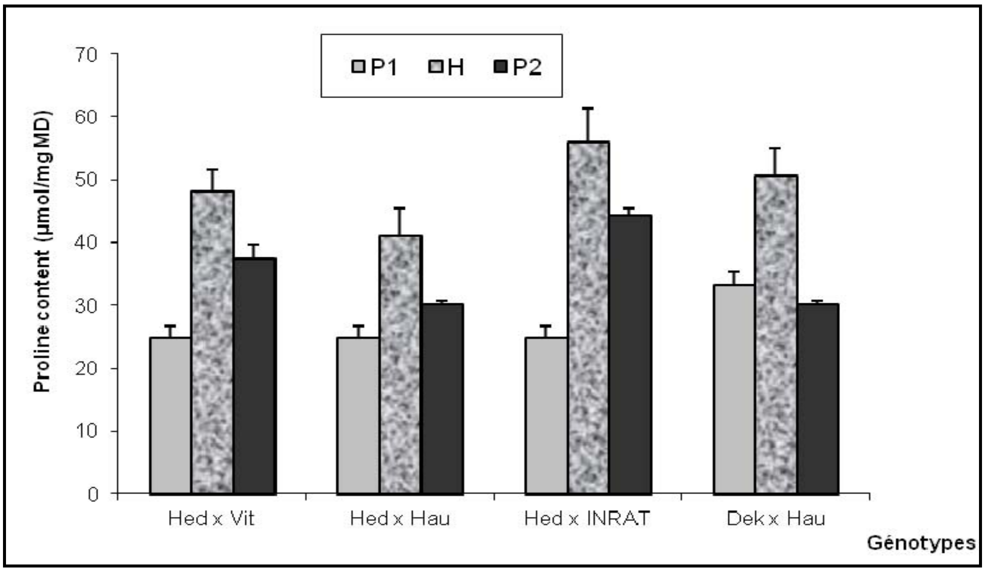

Figure 5. Leaves proline content in nine durum wheat genotypes (parents and F1 hybrid) under treatment of 6.5% FC.

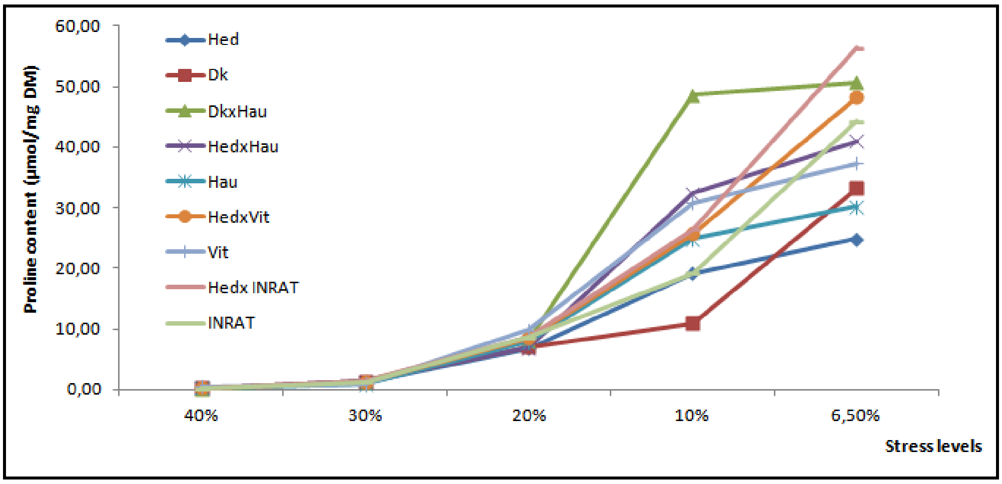

Figure 6. The evolution of the proline content in the F1 progeny at different levels of stress.

The statistical analysis of results obtained reveals the existence of a highly significant difference between the levels of stress on the one hand and between the genotypes on the other hand at the threshold 5% (Table 3).

The comparison between the proline content vis-à-vis the genotypes indicates that the levels, the larger are observed in the four F1 hybrids (Dk x Hau, Hed x INRAT, x Hed x Vit and Hed x Hau) into three groups (A, B and BC) with averages 21.76, 18.54 and 16.78 to 16.41 µmol / mg DM successively. While the biological parents are five levels of intermediaries, the first two INRAT Lives and record levels close to the hybrid groups (BC and CD) with averages 15.91

and 14.68 µmol / mg DM. While the other three (Hau, Dk and Hed) fall into groups (DE and E) with mean (12.83, 10.57 and 10.42 µmol / mg DM) respectively.

Table 3. Analysis of variance of the proline content in the F1.

Source of variance	DDL	CM	Test F	P	C.V	Signification
Total Variance	134	296.82				
Genotypes Var.	8	204.84	20.55	0.0000		***
Stress Var.	4	8406.7	843.44	0.0000		***
.Inter F Var	32	112.85	11.32	0.0000		***
Résiduelle1 Var.	90	9.97			20.66	

Changes in levels for the different treatments (levels of applied stress) reveals four groups: A> B> C> D ⇔6.5%> 10%> 20%> 30%, 40 ⇔40.69> 26.43> 08.06> 1.21; 0.26.

Stomatal resistance

Stomatal resistance was slightly lower at 40% and 30% FC (Figure 7). It varies from 33.95 ± 0.86 m^2 s/mol at Hed x INRAT to 177.12 ± 0.66 m^2 s/mol in Vit. It decreases to 30% FC for all genotypes except Hed x INRAT and INRAT. It has 4.36 and 2 times the initial value at F.C 40% respectively. At moderate stress 20% FC, stomatal resistance increased in all genotypes except the two varieties Hed and Vit. This increase is in the range of 1.16 to 2.72 times the initial value recorded at 40% FC. In severe stress 10% FC and 6.5% FC, stomatal resistance reached optimal values ranging from 331.5 ± 0.1 m^2 s/mol at Hed x Vit at 428.31 ± 0.1 m^2 s/mol at Hau. It is in the range of 2 to 4.28 times baseline to 40% FC except in two genotypes Hed x INRAT and INRAT it is almost 7 and 12 times the base value to 40% F.C.

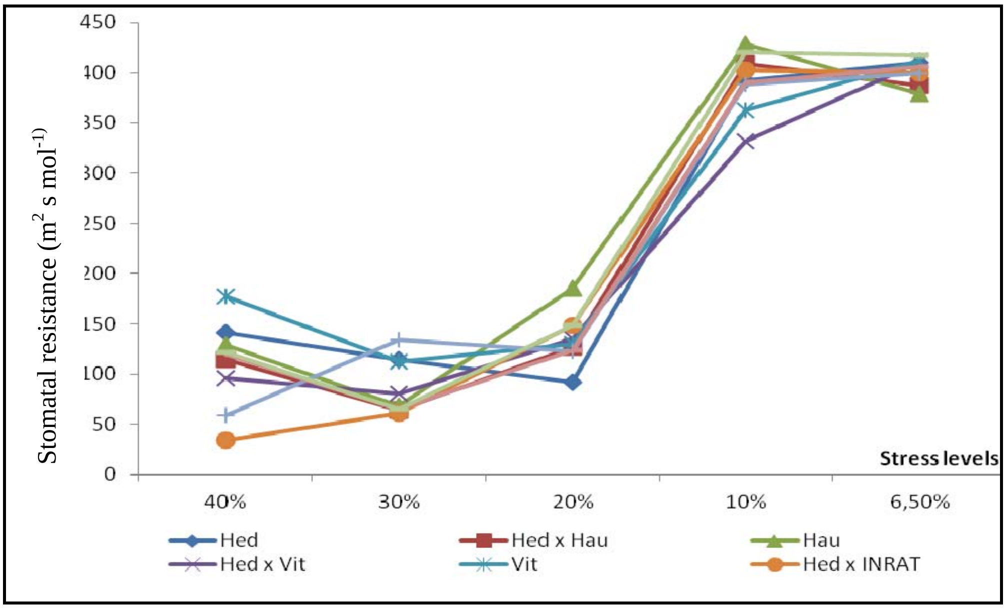

Figure 7. Changes in stomatal resistance of leaves of nine genotypes of durum wheat subjected to different levels of stress.

Kuruvadi's work (Kuruvadi, 1989) on wheat has shown that it responds to water stress by reducing the density of stomata. The decrease in the density of stomata does not always result in a reduction of water loss by the plant, because of its compensation by increasing their size (Sapra *et al.*, 1975; Wang and Clarke, 1993). The prolonged closure of the stomata results in the cessation of photosynthetic processes and the deaths of most of the plants as if our experience

6.5% CC Rapid stomatal closure as a better adaptation to drought, allowing the plant to conserve water available and to maintain a water content of tissues and also a high sensitivity to dehydration. Instead a slower stomatal closure may reflect a tolerance to dehydration, which may be accompanied by an osmotic adjustment. The speed of response of the stomata over time not only depends on the species and their water consumption but also water reserve land use (Rejeb *et al*., 1991).

The statistical study reveals a very highly significant difference for the factor stress. As against it does not reflect any significant difference between the nine genotypes studied.

Table 4. ANOVA of stomatal resistance in the F1.

Source of variance	DDL	CM	F-test	P	ET	C.V	significance
Total Variance	51	2298.6					
Genotypes Var.	3	2773.00	1.02	0.0000			***
Stress Var.	12	907.84	0.33	0.7116			NS
Inter F Var	36	2722.86			33.26	13.58	
Résiduelle1 Var.							

Newman Keuls test indicated three homogeneous groups for the factor stress: A> B> C ⇔ 6.5%, 10%> 20%> 40%, 30% ⇔ 402.58> 391.83> 134.55> 110.24> 84.84.

The CO_2 content, temperature, photoperiod and relative humidity of the air are factors as important as the water deficit and light, which also influence the functioning stomatal (Bezzala, 2005). The ability to maintain open stomata appears to be a mechanism of resistance adopted by the genotypes studied to cope with water stress. Thus, sensitive stoma genotypes are recommended for environments characterized by short-term droughts. Hence the use of stomata insensitive genotypes would be more beneficial (Raissac, 1992).

Conclusion

The accumulation of proline is positively correlated with the degree of stress. The four F1 hybrids exhibit high levels relative to their biological parents in a gain of maximum heterosis reached up to 100% in the hybrid Dek x Hau. Results obtained with 6.5% treatment really argue the water stress effect on the accumulation of proline. The most significant concentrations are observed in F1 hybrids, while the parents take intermediate levels.

The stomatal resistance increases with the degree of stress and actually manifests itself only at the 10% treatment of F.C. it appears, contrary to the results of several authors that tolerance to water shortage in cereals is not associated with isolated physiological behavior of the variety but with a strategy that includes one or more mechanisms of tolerance and/or avoidance of stress water. Due to the unpredictability of water stress, the best strategy would be one that allows the variety to present a wide range of adaptation.

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